NIST Develops Reference Methods and Standard Reference Materials for Clinical Diagnostic Markers

For nearly four decades NIST has been involved in the development of reference methods and SRMs to support accuracy in health-related measurements. In the 1960s NIST developed pure compound standards for use as calibrants. During the 1970s the focus was on the development of highly accurate and precise isotope dilution mass spectrometry-based "definitive methods" for 12 health status markers (i.e., Ca, Cl, Li, Mg, K, Na, cholesterol, creatinine, glucose, triglycerides, urea, and uric acid) in human serum. The first human serum-based SRMs certified for metabolites and electrolytes (e.g., SRM 909 Human Serum) were introduced in the 1980s. During the 1990s new serum-based SRMs were issued as frozen rather than lyophilized materials, and new efforts were focused on proteins as health markers as well as toxic metals. By the beginning of the 21st century, these efforts were directed toward the development of reference measurement procedures and reference materials for new health status markers to address the European Union directive regarding in-vitro diagnostic medical devices (IVD MD) which requires that **"…..the traceability of values assigned to calibrators and control materials must be assured through available reference measurement procedures and/or reference materials of a higher order ..."**.

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Since 2000 NIST has developed reference measurement procedures (the preferred term, replacing definitive methods) and reference materials for several new health markers. New methods based on liquid chromatography/mass spectrometry (LC/MS) or tandem mass spectrometry (LC/MS/MS) have been developed for homocysteine, folate, estradiol-17β, cortisol, progesterone, testosterone, thyroxine (T4), and triiodothyronine (T3).

With the completion of the reference measurement procedures for these new markers, several new SRMs have been added to the suite of materials for clinical laboratory measurements. SRM 1955 Homocysteine and Folate in Human Serum was completed in 2006. Homocysteine is a risk factor for heart disease and other conditions associated with oxidative stress; folate counteracts effects of homocysteine and has also been shown to reduce the risk of neural tube defects in fetuses. This SRM is a three-level frozen serum material with certified concentrations for homocysteine and 5-methyltetrahydrofolic acid, the principal form of folic acid in blood and an important antioxidant. The development of SRM 1955 was a collaborative effort between NIST and the Centers for Disease Control and Prevention (CDC), both of which provided measurements for certification. SRM 971 Hormones in Human Serum is another new reference material and is intended to support measurements of the following hormones: estradiol-17β, cortisol, progesterone, testosterone, T3, and T4. The SRM consists of two serum pools, one from normal adult males and one from normal, premenopausal adult females. Measurements of cortisol and progesterone are completed, and the NIST results will be combined with results from two collaborating laboratories to assign certified values for these two hormones. Measurements of the remaining hormones will be completed in 2007.

The incidence of kidney disease is rising rapidly in the U.S. Early detection and treatment can prevent kidney failure, but early detection depends on better measurements of kidney function than are currently available. Serum creatinine is the preferred measurement, but existing methods provide varying results.

NIST collaborated with the National Kidney Disease Education Program and the College of American Pathologists to develop a new reference material, SRM 967 Creatinine in Frozen Human Serum, to address measurement variability among clinical methods.

As part of this work, NIST has developed a new, rapid, isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) method for serum creatinine to replace the tedious isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) definitive method used previously at NIST.

NIST also initiated development of a series of serum-based SRMs for assessment of nutritional status, including vitamin D, vitamin B_6, and vitamin B_{12}. The reliability of clinical laboratory measurements for these analytes has been called into question, and individuals suffering from nutritional deficiencies may not be properly identified and treated. NIST is working with the National Institutes of

Health (NIH) to develop reference materials to address measurement variability in this area.

Metabolomics is an emerging discipline that seeks to characterize the complex profile of metabolites in cells, tissues, and other biological samples. Because metabolite levels can provide insight into the response of an organism to disease, metabolomics research may lead to the discovery of new clinical markers for disease diagnosis and treatment. NIST is developing SRM 1950 Metabolites in Human Plasma to support the development of new measurement technology in metabolomics. SRM 1950 will consist of a plasma pool collected from 100 men and women (equal numbers of each) and with a racial distribution that reflects the U.S. population. The initial value assignment phase for this SRM will target approximately 50 metabolites for which NIST has existing methods (e.g., cholesterol, electrolytes, glucose, hormones).

These new reference methods and the associated SRMs will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements. Better clinical measurements lead to better diagnoses, enabling earlier and more cost-effective treatments.

Much of the focus in the field of proteomics is also directed toward identification of biomarkers for particular diseases. The success of this effort depends upon validated approaches to protein identification and quantification. NIST is continuing research to develop reference methods for clinically relevant proteins. One of the most promising approaches is to break the protein down into specific peptides and measure the concentration of the peptides. This "bottom-up" approach relies on quantitative digestion of the proteins with enzymes such as trypsin. NIST has been investigating the effects of experimental factors on trypsin digests of proteins and exploring the quantitative nature of tryptic digestion. Additionally, using *in vitro* methods for protein production, isotopically labeled proteins are being prepared for use as internal standards in quantitative methodology.

To address needs in healthcare and public safety, CDC has established a network of laboratories to monitor arsenic poisoning throughout the U.S. by measuring arsenic species in urine. To ensure accuracy of these measurements, CDC collaborated with NIST to produce SRM 2669 Arsenic Species in Frozen Human Urine. SRM 2669 will provide the accuracy base for biomonitoring of arsenic exposure. Concentrations of seven arsenic species (arsonite, arsonate, monomethylarsonate, dimethylarsinate, trimethylarsine oxide, arsenobetaine, and arsenocholine) indicative of different sources of arsenic exposure will be certified.

Recent state-of-the-art measurements of cadmium, mercury, and methylmercury in SRM 966 Toxic Metals in Bovine Blood were used to upgrade the certification status of the material. This work considerably enhances the quality of the SRM and its usefulness to the clinical measurement community, especially for speciated mercury measurements. The updated Certificate of Analysis now has certified values for lead, cadmium, and total mercury in both Levels 1 and 2, and reference values for inorganic mercury and methylmercury in Level 2. Prior to this work, methylmercury was only listed on the Certificate as an information value.

In addition to its public safety role, CDC collects data on the trace element content of human body fluids and tissues as part of the National Health and Nutrition Examination Survey (NHANES). NIST has characterized two biological materials for elemental composition: SRM 1598a Animal Serum and SRM 1577c Bovine Liver. Certified or reference values are provided for all of the elements that CDC monitors as part of NHANES.

Future plans: NIST is collaborating with the CDC to develop a new SRM to meet the expanding needs for the measurement of trace elements in human urine. This new SRM, which will be frozen urine, will include both a natural and a fortified level, and will be an improvement over the existing SRM 2670a Toxic Elements in Freeze-Dried Urine in terms of commutability and elemental composition.